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SURVIVAL OF MEOR SYSTEMS IN POROUS MEDIA

Topical Report

By
Rebecca S. Bryant
Jonell Douglas

March 1986

Work Performed Under Contract No. FC01-83FE60149

National Institute for Petroleum and Energy Research
Bartlesville, Oklahoma

National Energy Technology Laboratory
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ABSTRACT

It was determined that many potential environmental hazards from MEOR technology are extensions of EOR environmental problems. One unique concern of MEOR is that interactions between injected microorganisms and molasses, and those microorganisms already present in the reservoir might pose novel problems. It appeared necessary to obtain information regarding the indigenous microbial flora and the survival characteristics of a MEOR system after it encounters simulated reservoir microbial conditions. We found from laboratory studies that adventitious microbial species present in the molasses overgrew several of the indigenous microorganisms in Berea sandstone cores and also overgrew the injected MEOR microorganisms.

From the results of these studies, we conclude that:

1) The introduction of nutrients into a petroleum reservoir could stimulate the growth of indigenous microorganisms.

2) Microorganisms present in injected non-sterile nutrients can overgrow both injected microbes and indigenous microorganisms.

3) Spore-forming bacteria cannot survive time periods in porous media of 4-20 weeks if there are other bacterial types present.

INTRODUCTION

Microbial enhancement of oil recovery (MEOR) is a technique with potential for obtaining more crude oil from partially depleted petroleum reserves and heavy oil deposits. MEOR processes generally consist of the injection of a microbial population and some form of nutrient, usually molasses. The microorganisms feed on the molasses, and produce carbon dioxide, other gases, acids, alcohols, and/or surfactants. These microbial products are expected to mobilize the crude and allow it to move more freely to the producing well.

The increase in MEOR field projects deems it desirable to determine if MEOR techniques will be hazardous to the environment. The intent of this report is to answer certain questions regarding the potential environmental hazards of MEOR processes. These questions are: 1) What are the potential environmental MEOR hazards? 2) What types of microorganisms will the injected MEOR system (microbes and nutrients) encounter in the reservoir? 3) What happens in porous media after the MEOR process in terms of: a) oil recovery, b) survival of the MEOR microorganisms, and c) survival of the indigenous microorganisms.

The use of microorganisms to recover more oil from petroleum reservoirs has been the subject of many recent reviews (1-3). The potential uses of microorganisms for oil recovery are considered mainly to be tertiary methods and would only be applied after primary and secondary methods have been used. Microbial enhanced oil recovery (MEOR) can involve several types of processes, including the production in surface facilities of biopolymers for mobility control, and biosurfactants for reducing the interfacial tension between oil and water. These microbial products are injected into the oil bearing rock through injection wells.

Another MEOR technique involves the direct injection of microorganisms into the reservoir (see figure 1). The *in situ* MEOR processes involve growth and metabolism of the injected microbes in the reservoir. This activity produces chemicals that can aid in oil release from the reservoir rock. The microbial production of carbon dioxide, hydrogen, and methane can result in reducing the viscosity of the oil, increasing its capability to flow. Surface active compounds are also produced by a few microbial species. These products can reduce interfacial and surface tension, and viscosity of the crude oil.

Acids, primarily short chain fatty acid types, and alcohols are also microbial products resulting from metabolism. Acids may dissolve carbonate rock in reservoir while alcohols may behave similar to surface active agents.

Although some of these microbial products appear to be useful for enhanced oil recovery, there will be no metabolic activity or products unless the microorganisms can tolerate the reservoir environment. Clark, et al. (4) made a study of the reservoirs in nine oil-producing states for their EOR potential using microbes. The key screening parameters they evaluated included: <10 percent salt, a pH of 4-9, permeability >75 md, API gravity >17°, and temperatures <75° C. Using these parameters they found that 54 percent of California reservoirs, 42 percent of Colorado reservoirs, 36 percent of Louisiana reservoirs, 29 percent of New Mexico reservoirs, 23 percent of Wyoming reservoirs, 21 percent of Texas reservoirs, 20 percent of Kansas reservoirs, 16 percent of Oklahoma reservoirs, and 5 percent of Mississippi reservoirs were amendable to MEOR.

At present, chemical and thermal EOR processes are the largest EOR contributors and account for about 5 percent of the total U.S. production. It is predicted that this number will reach 30 percent by 1991. Likewise MEOR field projects have also exhibited an increase and could be expected to significantly contribute to the overall EOR production within the next decade.

Two of the major environmental concerns associated with MEOR field technology are: 1) possible contamination of surface and groundwater and 2) possible contamination of agricultural land. These concerns are also important for other EOR processes. Because of the increasing number of field tests using microorganisms, and since MEOR processes are often performed on shallow, abandoned, or stripper wells that are more likely to have corroded equipment and sub-surface tubing, the environmental consequences of MEOR technology should be assessed.

There are three main concerns associated with releasing microorganisms and/or nutrients into the environment. First, microorganisms have a rapid mutation rate. Should they mutate to some form that is pathogenic, or that produces harmful compounds, environmental problems will result. Some microorganisms are not pathogenic in air but if introduced into the body (by ingestion or through a cut, for example), they cause disease. Second, many microorganisms present in the reservoir can produce hydrogen sulfide, a gas

that is fatal to man. If nutrients stimulate the growth of these bacteria "souring" of the crude oil, or even poisoning of surrounding life could result. Third, there is the problem of seepage or leakage of the introduced microorganisms and/or nutrients into freshwater aquifers. The interactions of these microbes with others could be disastrous.

Little is known about the types of microorganisms that are present in petroleum reservoirs. Before microorganisms and nutrients are injected into a reservoir, there are several questions regarding their interactions that should be answered. If microorganisms or nutrients stimulate indigenous microbial flora to produce hydrogen sulfide or copious amounts of slime, the environmental problems may negate the economics of the MEOR process. Should deliberate introduction of microorganisms cause contamination of groundwater or surface water, the problem may go unnoticed for several years. Before applying MEOR technology the oil industry should know more about MEOR processes and their environmental consequences.

MATERIALS AND METHODS

To assess the MEOR technology, literature reviews were conducted during the first year of this project (2). This study indicated that, although many investigators are researching MEOR techniques, no environmental research relating to MEOR was found. Other pertinent MEOR research areas that merit interest include: data on how biosurfactants compare with synthetic surfactants under reservoir conditions, techniques for bio-emulsification of oil within the formation, modifications to increase the salt and heat tolerance of biopolymers in the reservoir, in situ mechanisms relating to transport, growth and metabolic product secretion of microorganisms under reservoir simulated conditions, and selective plugging by microorganisms in situ for mobility control.

A data base for MEOR has been designed and developed at NIPER. There are actually two data files for this data base. One is a data file containing information about the microorganisms used for MEOR and the other data file contains the field information about MEOR field tests. For some entries, very little information has been available while other entries contain all the necessary information regarding the MEOR field test and the microorganisms used. A scrutiny of this data base will show that publications on field technologies, scale-up processes, and economic data are nonexistent.

Microorganism Selection

Approximately ten petroleum reservoir-produced water samples were examined for predominant bacterial types. About fifty percent of the isolates were Bacillus species (Gram-positive, spore-forming rods), and thirty percent were Gram-negative rods (strictly aerobic or facultatively anaerobic). The types of bacteria that were commonly found in the majority of the water samples were designated as "indigenous microorganisms." These were subjected to several biochemical tests for further characterization (table 1). Fifty percent of these selected microorganisms can ferment sucrose and eighty-five percent can grow and metabolize in five percent sodium chloride. For the experiments, three Bacillus isolates were selected (H-1, H-2, H-3) and three Gram-negative facultatively anaerobic rods (H-12, H-13, G-2) were chosen to combine with the Bacillus strains. This selection gave us two indigenous

microorganisms per core that could easily be distinguished by microscopic observations.

A species of Clostridium (a Gram-positive spore-former and strict anaerobe) or Bacillus (a Gram-positive spore-former and faculatative anaerobe) was chosen for the simulation of the MEOR process. These two microbial genera are currently being used in field tests in Oklahoma, Kansas, and Texas (5) and were provided by Oklahoma State University.

Preparation of Apparatus

Cylindrical Berea sandstone cores, supplied by Cleveland Quarries, Amherst, Ohio, were fired at 800° F for 24 hours to stabilize the clays. The cores were 10 inches long by 1.5 inches in diameter, and were sealed in an epoxy resin to confine the flow of injected fluids to a linear path. The cores were evacuated and filled with 0.5 percent NaCl brine. The absolute permeability to brine ranged from 257 millidarcies to 376 millidarcies, and the average permeability was 311 millidarcies (table 5). The pore volume of the cores ranged from 57.3cc to 60cc and average 58.2cc. The cores were crude oil flushed until no additional water was produced to simulate connate water saturation. The oil saturated cores were flooded with 0.5 percent NaCl brine prior to microbial injection. This reduces oil saturation to simulate residual oil saturation after a waterflood. The percent of residual oil in the core ranged from 25.08 percent to 38.55 percent and the average was 34.0 percent. Figure 2 illustrates the experimental apparatus. The fluid separators are piston devices used to inject microbial solutions and other fluids into the cores. They are of Lexan material and were constructed at NIPER. These devices were designed to prevent microorganisms and crude oil from passing through the pumps.

Chemicals and Media

All chemicals and bacteriological media were obtained from Sigma Chemical Company and Curtin Matheson Scientific, Inc. The crude oil used in these experiments was obtained from the Bartlesville sand in the Delaware-Childers field in northeastern Oklahoma. Its specific gravity is 0.851 at 25° C and its viscosity is around 10 centipoises. The molasses used was Mr. Blackstrap 87. The composition is as follows: crude protein - 5 percent; crude fat -

0.5 percent; and total sugars - 38 percent. Its final concentration was 4.0 percent by weight in water, after filtration through cheesecloth.

Experimental Protocol

Figure 3 illustrates the flowchart used for the MEOR-indigenous microbial flora experiments. The selected indigenous microorganisms were cultured in pairs in anaerobic tubes of trypticase soy broth (TSB). After an incubation period of 48 hr at 37° C, the tubes were examined for the following: 1) relative numbers of Bacillus species and Gram-negative rod species; 2) endospore formation by the Bacillus species; and 3) whether the addition of Delaware-Childers crude oil and Berea sandstone altered the relative ratio of the two species. It was observed that there were certain combinations of bacteria that could multiply in basically a 1:1 ratio, while others could not survive at this ratio, without one overgrowing the other. The pairs H-2 and H-12, H-3 and H-13, and H-1 and G-2 were selected for the experiments.

Each residual oil-saturated Berea sandstone core was injected with one pair of the indigenous microorganisms. After an incubation period of one week at 37° C, a sample of the effluent from the core was plate-counted on trypticase soy agar and microscopically examined. Clostridium (approximately 1×10^6 cells/ml) in molasses was injected into the core (0.5 pore volumes (PV), and the core was incubated for another week at 37° C, or for several weeks at ambient temperature. The Bacillus species was also injected in the same manner. The indigenous microbial population was not used for the Bacillus experiments. The cores were waterflooded (0.5 percent NaCl) after the time period, and effluents were plate counted and microscopically examined. The parameters observed were: oil recovery (Re) by the MEOR system, total number of microorganisms in the effluent, identification of the microorganisms in the effluent, and gross morphological or biochemical changes in characteristics of the microorganisms.

RESULTS AND DISCUSSION

What are the potential environmental MEOR hazards?

There are several environmental impacts that are associated with MEOR field projects.

1) Possible stimulation of sulfate-reducing bacteria indigenous to the reservoir - These bacteria may produce hydrogen sulfide, which could sour the crude oil and be released as a gas potentially hazardous to oilfield personnel.

2) Stimulation of other indigenous microorganisms which could produce slime and plug the equipment lines, or oxidize ferrous salts to ferric hydroxide, causing corrosion of casing.

3) Possible contamination of the crude oil itself and associated gases and water, foam, emulsions formed with the produced oil.

4) Possible contamination or degradation of other EOR chemicals used later or concurrently with the MEOR process.

These environmental impacts are some of the negative aspects of MEOR processes; however, there are favorable environmental impacts that are addressed below:

1) Better use of environmental resources for increased oil recovery.

2) MEOR processes, if applied properly, are more easily controlled systems within the reservoir. The injected microorganisms cannot multiply in the reservoir environment unless nutrients are supplied. Therefore, if the nutrients are stopped, the system will stop. This decreases the need for chemicals or processes with more potential for harming the reservoir environment. Once a chemical is injected, it can migrate, while microorganisms must be driven or fed to effect this environmental impact.

3) MEOR processes consume less energy that other EOR methods such as steam, thermal, etc.

4) A potential for using environmental polluting wastes as nutrients for MEOR processes exists, creating a safer method of their disposal.

A total of eighteen core experiments were used to evaluate the longevity of MEOR systems and interactions with indigenous microbial flora. Table 4

identifies the core, what, if any, indigenous microorganisms were used, whether Clostridium or Bacillus was injected for the MEOR process simulation, and the incubation period in weeks for each core.

Bacillus Cores

Five cores were injected with Bacillus and molasses. Core M-17 served as the control experiment, and received autoclaved (121° C; 15 min) Bacillus cells and non-sterile molasses. The total cell numbers of the Bacillus core effluents differed by two orders of magnitude (1.5×10^4 to 3.84×10^6 cells/ml; table 3). Graphs of the plate count results (figure 4) did not indicate a trend in the numbers of cells present in the effluents. The cell numbers did not decrease significantly at the outlet core face. The most significant finding regarding the Bacillus cores is that only in the 4-week incubated core, M-22, was Bacillus species found in the effluent. The Gram-variable rod contaminant was found to be present in all effluents, and was assumed to be a contaminant from the non-sterile molasses. Since Bacillus forms spores (resistant cell structures more impenetrable to environmental stresses) it was logical to assume that it would have the best survivability under reservoir conditions.

Clostridium - One-Week Incubation

All but two of these cores had the total number of cells in the effluent between 1.0 and 5.0×10^5 cells/ml. The other two cores, M-9 and M-16, and the same two indigenous microorganisms injected (H-3 and H-13). Both of these effluents had plate counts ten to one hundredfold lower than the other core effluents. The Clostridium species injected was present in all core effluents after one week. The C* molasses contaminant was also found in all effluents. When indigenous microorganisms were injected (M-9 through M-16), in no instance did the injected Bacillus species survive. In all core effluents, the Gram-negative rod (GNR), as well as C*, was found.

Clostridium - Long Term Incubation

The total number of cells from core effluents that had Clostridium and molasses injected did not appear to follow any set pattern (figure 6). The cores that had indigenous microorganisms injected before the Clostridium and

molasses (M-9a, M-10a, M-11a, M-15a, M-16a) all had approximately the same number of cells. The core effluents differed from the one week incubation effluent plate counts because cores M-9a and M-16a showed plate counts that were as high as the other cores. In the one-week experiments, the counts were ten to one hundredfold lower for these two cores.

Only two core effluents contained the injected Clostridium species, cores M-8a and M-10a. M-8a did not receive any indigenous microorganisms; however, M-10a had indigenous microorganisms H-2 and H-12 colonizing the core. The plate counts did not differ significantly for M8a or M10a.

All core effluents contained C*, the molasses contaminant that was also found in all other core effluents with non-sterile molasses injected. In one experimental core, M-7, Clostridium and sterile molasses was injected, and the C* was not observed. This again indicates that the source of C* is the molasses.

Recovery Efficiencies

Figure 7 illustrates the recovery efficiencies of the Bacillus and Clostridium MEOR systems (table 5). The number of weeks that the cores incubated seems to have little effect on the recovery efficiencies of the Bacillus system. It is interesting to note that both 12-week core experiments (M-1 and M-3) had almost identical recovery efficiencies. The successful repetition of this experiment lends support to the overall recovery efficiency data. M-17 had only non-sterile molasses and autoclaved Bacillus, and its recovery efficiency was only 5 percent, making it a control core.

Since the Clostridium cores were all incubated for one week, comparisons can only be made with different indigenous microorganisms used in each core. The absence of indigenous microorganisms in the cores during the Clostridium and molasses injection increased the recovery efficiency of the system. The degree of increase is not consistent; however, since the recovery efficiency ranges from 21.43 percent (M-11) to 13.54 percent (M-15), while the core without indigenous microorganisms had a recovery efficiency of 23.0 percent (table 5). Another interesting finding from these experiments was that the two lowest recovery efficiencies were from the two cores that had H-2 and H-12 colonized. However, no relationship between the indigenous microbial pairs could be found. The difference in recovery efficiency between M-7 that had

sterile molasses, and M-8 with non-sterile molasses is the greatest of the Clostridium cores. This indicates that the contaminating microorganisms in the non-sterile molasses could be aiding in the oil recovery efficiency.

What types of microorganisms will the MEOR system encounter?

Results of work the past two years (FY84 and FY85) has shown that in the produced waters of tested reservoirs in Oklahoma, Kansas, and Texas, about fifty percent of the microorganisms are Bacillus species, while the other half are about forty percent Gram-negative facultatively anaerobic rods, and about ten percent Gram-positive cocci (in the more shallow wells). These are considered to be representative indigenous microbial flora from petroleum reservoirs having comparable characteristics of low salinity, depths of less than 3000 feet, and sandstone rock composition. There are many reports indicating that sulfate-reducing bacteria would be present in some petroleum reservoirs (6).

What happens in porous media after the MEOR process?

Bacillus MEOR System

The cores containing the Bacillus species as the MEOR simulated process were incubated for periods ranging from four to twenty-one weeks, and the plate counts stayed relatively the same for all core effluents. It appears that some microorganism can survive up to twenty-one weeks under reservoir conditions and still maintain relatively the same population count. It was a surprise to learn that the Bacillus species, however, could not survive incubation periods longer than four weeks. The non-sterile molasses introduced another microorganism that out-competed the injected Bacillus species. It is unknown at this time whether a core using Bacillus and sterile molasses would have shown the influence of C* more clearly in these studies. Since we were interested in the MEOR process as it would be applied in the field, non-sterile molasses was used.

The ability of another microorganism to overtake spore-forming microbes could affect the efficiency and success of an MEOR field project. When non-sterile molasses and killed Bacillus cells were injected, the recovery

efficiency was very low (5 percent) while the recovery efficiencies of the Bacillus and non-sterile molasses averaged a figure at least two times higher.

Clostridium - One-week Incubation

Two microbial species can be successfully colonized in an oil-saturated Berea sandstone core. By using microscopic observations and plate counts, it was found that they maintained the same relative ratio to each other (1:1) as before any other microorganism or molasses was introduced (7). The injection of Clostridium and non-sterile molasses changed the ratio of the two indigenous microorganisms, and in all cases, the Bacillus species did not survive, while the C* from the non-sterile molasses was present in all core effluents. The Clostridium species injected was also present in all core effluents.

We conclude that the Bacillus species indigenous to the reservoir would probably be overtaken by contaminants in the molasses or other nutrients injected. They could also be overgrown by stimulation of the Gram-negative facultatively anaerobic rods introduced to the reservoir by the molasses. The disappearance of Bacillus may or may not have an adverse effect on the reservoir environment. If the microorganisms were producing beneficial products required for the growth of other microbial species, or if the Bacillus species was degrading some important environmental pollutant, then obviously it would not be desirable to wipe out the Bacillus population. However, it may not be contributing at all to the reservoir environment, and thus not be considered an environmental impact.

Clostridium - Long Term Incubation

The results of these experiments corroborated the results of the short term (one week) Clostridium experiments. The plate counts from the twenty-week core (M-8a) were comparable to the plate counts from the twelve, fourteen, and sixteen-week cores. The introduction of the indigenous microorganisms appeared to affect the survival of the Clostridium species. Clostridium was present in only one effluent with indigenous microorganisms (M-10). In the core with only Clostridium and non-sterile molasses injected, the Clostridium survived for twenty weeks, although the C* contaminant from the molasses was also present. Again, as in the other cores, the injected

Bacillus species did not survive; however, in these longer term incubations, the Gram-negative rod (H-12, H-13, G-2) also did not survive.

The same conclusions can be stated for this series of experiments. The introduction of nutrients such as microorganisms in a MEOR field test may suppress the indigenous microorganisms flora in the reservoir. Additionally, it appears that Clostridium has a much better chance of survival under reservoir conditions of sandstone rock, temperatures of 40° C, and low salinity (0.5 percent NaCl), than does the Bacillus species.

The results of these studies show the importance of determining the indigenous microbial population of a reservoir before an MEOR process is applied. The injection of nutrients could stimulate an indigenous population to grow and the results would be beneficial or disastrous. The nutrients may serve to stimulate an in situ MEOR process, thereby improving oil recovery, or alternatively the nutrients could stimulate an indigenous microbial population that produces slime and plugs up the reservoir. Microorganisms present in non-sterile nutrients can overtake both injected and indigenous microorganisms. Again, this activity may serve to enhance an MEOR process, or it may shut the whole system down, depending upon the microorganisms involved. Spore-forming bacteria have long been assumed to be the most capable of survival under stressful conditions such as heat, dryness and ionic strength. We conclude that some spore-forming bacteria will not be useful in an MEOR process if there are other microorganisms present in the reservoir or in the molasses that can suppress growth of the spore-former. This knowledge can be useful to operators planning MEOR field projects, and if at all possible, the field operators must work with microbiologists to determine the best MEOR process for that field.

CONCLUSIONS

1. The introduction of nutrients into a petroleum reservoir could stimulate the growth of indigenous microorganisms as indicated from core experiments.

2. Microorganisms present in injected non-sterile nutrients can overgrow both injected microbes for EOR and indigenous microorganisms as has been determined from laboratory core experiments.

3. Spore-forming bacteria injected in cores did not survive time periods up to 20 weeks if there are other bacterial types present.

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TABLE 1. - Microbial isolates from petroleum reservoirs.

Code	Source	O_2 Requirement	H_2S Producing	Gram Stain and Morphology
H-1	Oklahoma well, 3000 ft	Facultative	-	GPR
H-2	Texas well, 2000 ft	Facultative	-	GPR
H-3	Oklahoma well, 3000 ft	Facultative	-	GPR
H-4	Kansas well, 1000 ft	Facultative	-	GPR
H-5	Oklahoma well, 2000 ft	Aerobic	-	GNR
H-6	Oklahoma well, 2000 ft	Facultative	-	GPR
H-7	Oklahoma well, 2000 ft	Facultative	-	GPR
H-8	Oklahoma well, 975 ft	Facultative	-	GNR
H-9	Oklahoma well, 975 ft	Facultative	-	GNR
H-10	Oklahoma well, 975 ft	Facultative	-	GPR
H-11	Oklahoma well, 975 ft	Aerobic	-	GNR
H-12	Kansas well, 1000 ft	Facultative	-	GNR
H-13	Texas well, 2000 ft	Facultative	-	GNR
H-14	Oklahoma well	Facultative	-	GPR
G-1	Bartlesville well, <600 ft	Facultative	-	GPR
G-2	Bartlesville well, <600 ft	Facultative	-	GNR
D-2	Bartlesville well, <600 ft	Facultative	-	GPR
D-4	Bartlesville well, <600 ft	Facultative	-	GPR
Bu-1	Butler Formation, <600 ft	Aerobic	-	GPR
Bu-2	Butler Formation, <600 ft	Aerobic	+	GNR
Bu-3	Butler Formation, <600 ft	Aerobic	-	GPR
Bu-A	Butler Formation, <600 ft	Aerobic	-	GPC
Bu-An	Butler Formation, <600 ft	Anaerobic	+	GPR
COS-1	Bartlesville Formation, 680 ft	Aerobic	-	GNR
COS-2	Bartlesville Formation, 680 ft	Facultative	-	GNR
COS-3	Bartlesville Formation, 680 ft	Aerobic	-	GNR

GPR = Gram positive rod

GNR = Gram negative rod

GPC = Gram positive cocci

TABLE 1. - Microbial isolates from petroleum reservoirs (continued).

Code	Spore Production	Sucrose Reaction	Salt Tolerance (5%)	Beta-Hemolysis
H-1	+	-	+	+
H-2	+	-	+	-
H-3	+	-	-	+
H-4	+	-	+	-
H-5	-	-	+	-
H-6	-	+	+	-
H-7	+	+	-	+
H-8	-	+/Gas	+	-
H-9	-	+/Gas	+	-
H-10	+	+	+	+
H-11	-	-	+	-
H-12	+	-	+	-
H-13	-	-	+	-
H-14	+	+/Gas	+	+
G-1	+	+/Gas	-	+
G-2	-	+/Gas	+	+
D-2	+	+/Gas	-	+
D-4	+	+/Gas	+	-
Bu-1	+	-	+	-
Bu-2	-	-	+	-
Bu-3	-	-	+	-
Bu-A	-	+/Gas	+	-
Bu-An	+	+/Gas	+	+
COS-1	-	-	+	-
COS-2	-	+/Gas	+	-
COS-3	-	-	+	+

+ indicates positive reaction

- indicates negative reaction

TABLE 2. - Core Experiments

Core Number	Indigenous Microorganisms[1]	Injected Microorganisms[2]	Incubation (wks)
M-1	-	Bacillus sp.	12
M-3	-	Bacillus sp.	12
M-5	-	Bacillus sp.	21
M-22	-	Bacillus sp.	4
M-17	-	Bacillus sp.[3]	16
M-7[4]	-	Clostridium sp.	1
M-8	-	Clostridium sp.	1
M-9	H-3, H-13	Clostridium sp.	1
M-10	H-2, H-12	Clostridium sp.	1
M-11	H-1, G-2	Clostridium sp.	1
M-15	H-2, H-12	Clostridium sp.	1
M-16	H-3, H-13	Clostridium sp.	1
M-8a	-	Clostridium sp.	20
M-9a	H-3, H-13	Clostridium sp.	12
M-10a	H-2, H-12	Clostridium sp.	12
M-11a	H-1, G-2	Clostridium sp.	14
M-15a	H-2, H-12	Clostridium sp.	14
M-16a	H-3, H-13	Clostridium sp.	16

1 A species of Bacillus (H-1, H-2, H-3) and a Gram-negative rod (G-2, H-12, H-13).

2 0.3 pore volumes of bacteria and 0.5 pore volumes of 4.0 percent molasses were injected each time.

3 This Bacillus species was autoclaved before injection with the molasses.

4 Sterile molasses was used in this experiment.

TABLE 3. - Aerobic Plate Counts and Observations

Core	Fraction[1]	Cells/ml	Comments[2]
M-1	1	4.79×10^5	No Bacillus, GNR
	2	1.53×10^4	" "
	3	1.57×10^5	" "
M-3	1	1.66×10^4	No Bacillus, GNR
	2	4.63×10^4	" "
	3	2.44×10^4	" "
M-5	1	3.84×10^6	No Bacillus, GPC, GNR
	2	1.25×10^5	" " "
	3	7.90×10^4	" " "
M-22	1	4.40×10^5	Bacillus, GNR
	2	5.40×10^5	" "
	3	4.55×10^5	" "
M-17	1	3.41×10^5	No Bacillus, GNR
	2	4.90×10^4	" "
	3	1.50×10^4	" "

1 Each fraction equals 10 ml core effluent (consecutively). Each fraction was diluted and pour plates prepared using TSA. Plates were incubated at 37° C for 24-48 hours.

2 GNR = Gram-negative rod
GPC = Gram-positive cocci

TABLE 4. Results of Core Experiments Using Mixed Cultures.

Expt M-9[2]

Fluid Fractions Produced (PV)	Total Counts Cells/ml	Numbers of Types of Microorganisms	Designation of Types[1]
Original	7.10×10^6		H-3 & H-13
0.1PV	1.87×10^4	2	H-3 & H-13
0.2PV	2.11×10^4	2	H-3 & H-13
0.3PV	3.93×10^5	2	H-3 & H-13
0.4PV	2.16×10^4	2	H-3 & H-13
0.5PV	1.34×10^4	1	H-13
After MEOR Process			
M-9	Fraction		
	1	1.67×10^3	C*,GNR,No Bacillus
	2	2.02×10^2	" " "
	3	3.85×10^4	" " "

Expt M-10[2]

Original	6.20×10^6		H-2 & H-12
0.1PV	4.40×10^5	1	H-12
0.2	3.61×10^5	2	H2 & H-12
0.3	3.05×10^5	1	H-12
0.4	4.24×10^5	2	H2 & H-12
0.5	3.34×10^5	2	H2 & H-12
After MEOR Process			
M-10	Fraction		
	1	3.06×10^5	C*,GNR,No Bacillus
	2	1.57×10^5	" " "
	3	5.34×10^5	" " "

[1]C* - Gram-variable contaminant

[2]Each core (one-week incubation) had Clostridium in the effluent.

TABLE 4. Results of Core Experiments Using Mixed Cultures (continued).

Expt M-11[2]

Fluid Fractions Produced (PV)	Total Counts Cells/ml	Numbers of Types of Microorganisms	Designation of Types[1]
Original	5.30×10^6		H-1 & G-2
0.1PV	3.32×10^5	2	H-1 & G-2
0.2	1.95×10^5	2	H-1 & G-2
0.3	2.98×10^4	2	H-1 & G-2
0.4	1.40×10^4	2	H-1 & G-2
0.5	1.20×10^4	2	H-1 & G-2
After MEOR Process			
M-11	Fraction		
	1	3.45×10^5	C*,GNR,No Bacillus
	2	4.40×10^5	" " "
	3	4.60×10^5	" " "

Expt M-15[2]

Original			
	5.7×10^5		H-2 & H-12
0.1	1.35×10^4	2	H-2 & H-12
0.2	2.47×10^5	2	H-2 & H-12
0.3	3.20×10^4	2	H-2 & H-12
0.4	2.50×10^5	2	H-2 & H-12
0.5	3.70×10^5	2	H-2 & H-12
After MEOR Process			
M-15	Fraction		
	1	3.30×10^5	C*,GNR,No Bacillus
	2	4.61×10^5	" " "
	3	3.43×10^5	" " "

[1]C* - Gram-variable contaminant

[2]Each core (one-week incubation) had Clostridium in the effluent.

TABLE 4. Results of Core Experiments Using Mixed Cultures (continued).

Expt M-16[2]

Fluid Fractions Produced (PV)	Cells/ml	Numbers of Types of Microorganisms	Designation of Types[1]
Original	4.20×10^6		
			H-3 & H-13
0.1	3.85×10^4	2	H-3 & H-13
0.2	4.16×10^4	2	H-3 & H-13
0.3	5.55×10^4	2	H-3 & H-13
0.4	4.23×10^5	2	H-3 & H-13
0.5	3.21×10^5	2	H-3 & H-13
After MEOR Process			
M-16	Fraction		
	1	1.13×10^4	C*,GNR,No Bacillus
	2	1.88×10^4	" " "
	3	3.17×10^5	" " "
M-7	Fraction		
	1	3.20×10^5	Clostridium, C*
	2	3.55×10^5	" "
	3	4.50×10^5	" "
M-8	Fraction		
	1	4.90×10^5	Clostridium, C*
	2	5.00×10^5	" "
	3	6.10×10^5	" "

[1]C* - Gram-variable contaminant

[2]Each core (one-week incubation) had Clostridium in the effluent.

TABLE 5. Results of Long Term Core Experiments for *Clostridium*

	Fraction[1]	Cells/ml	Designation of Types[2]
M-8a	1	1.88×10^6	*Clostridium*, C*[2]
	2	1.02×10^6	" "
	3	6.60×10^5	" "
M-9a	1	4.62×10^5	No *Clostridium*, C*
	2	1.24×10^5	" "
	3	1.88×10^4	" "
M-10a	1	6.77×10^5	*Clostridium*, C*
	2	4.43×10^5	" "
	3	8.20×10^4	" "
M-11a	1	4.34×10^4	No *Clostridium*, C*
	2	3.72×10^4	" "
	3	7.64×10^4	" "
M-15a	1	8.78×10^5	No *Clostridium*, C*
	2	1.00×10^5	" "
	3	1.81×10^5	" "
M-16a	1	9.62×10^5	No *Clostridium*, C*
	2	8.90×10^5	" "
	3	4.74×10^5	" "

[1]Each fraction equals 10 ml core effluent (consecutively)

[2]C* = Gram-variable molasses contaminant

TABLE 6. - Recovery Efficiencies of Bacillus and Clostridium

Core	Sowf (%)	Socf (%)	Recovery Efficiency (%)	Pore Vol (cc)	K mD
Bacillus[1]					
M-1	25.08	22.54	10.14	59.0	257
M-3	30.60	27.52	10.07	58.5	309
M-5	26.83	21.92	18.30	60.0	305
M-22	36.13	30.71	15.00	57.3	326
AVERAGE			13.38%		
Sterile Molasses					
M-7[1]	32.42	30.88	5.04	58.1	330
Clostridium					
M-8[1]	36.90	28.40	23.00	57.7	339
M-9	37.07	30.86	16.70	58.0	315
M-10	34.66	29.46	15.00	57.7	287
M-11	38.55	30.29	21.43	58.1	376
M-15	38.40	33.28	13.54	58.6	273
M-16	37.33	29.51	20.47	57.6	331
AVERAGE			18.35%		

[1] No indigenous microorganisms were injected prior to the MEOR process.

K = Absolute permeability, in millidarcies

$$\text{Recovery Efficiency} = \frac{\text{Sowf} - \text{Socf}}{\text{Sowf}}$$

Sowf = Residual oil saturation after waterflood

Socf = Residual oil saturation after microbial treatment

CYCLIC MICROBIAL RECOVERY

A solution of microorganisms and nutrients is introduced into an oil reservoir during injection. The injection well is then shut in for an incubation period allowing the microorganisms to produce carbon dioxide gas and surfactants that help to mobilize the oil. The well is then opened and oil and products resulting from the treatment are produced. This process may be repeated.

Schematic portrays one well during the 3 phases of this process. Flow pattern is stylized for clarity.

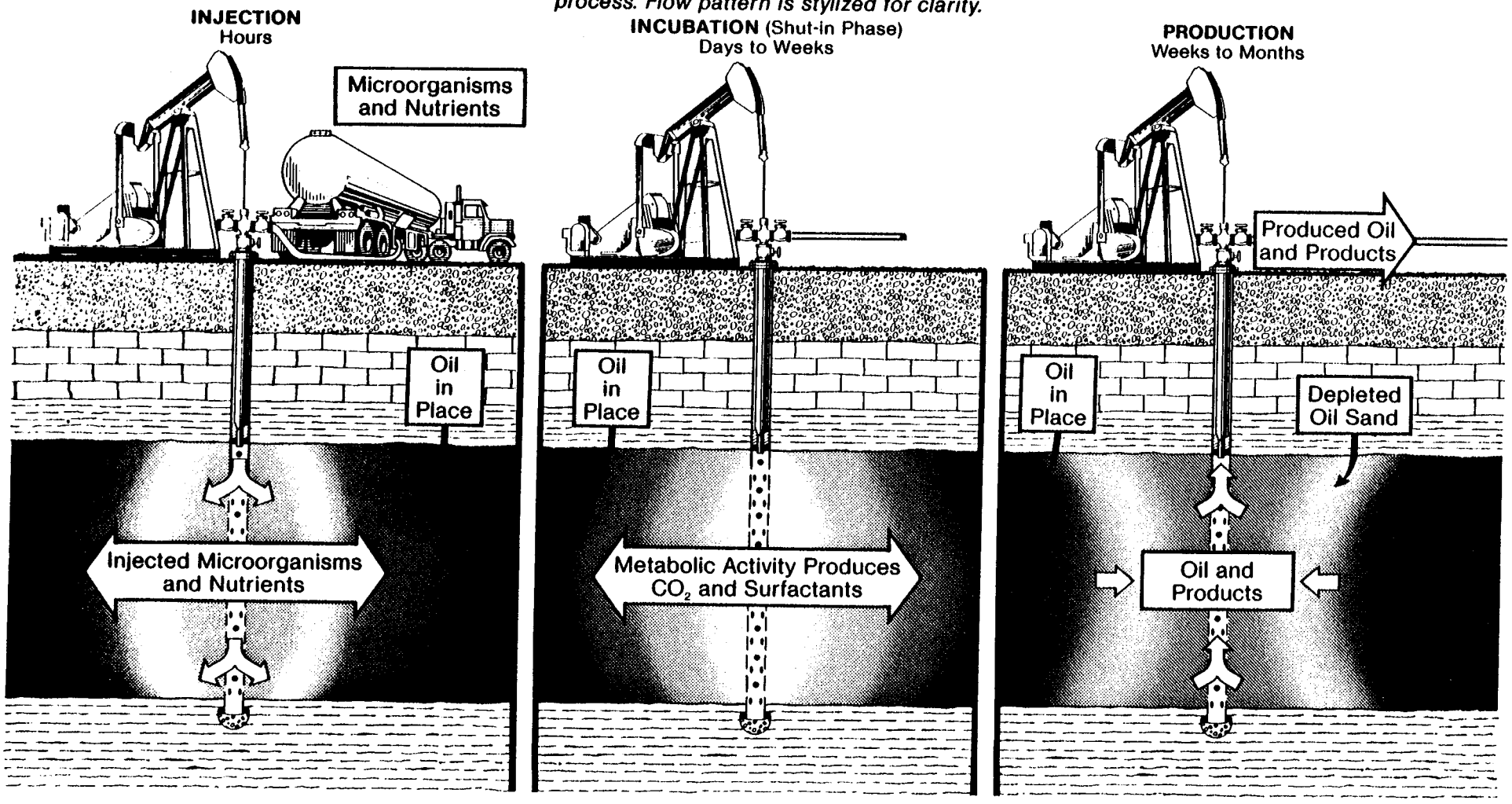

FIGURE 1. - Direct injection of microorganisms into the reservoir.

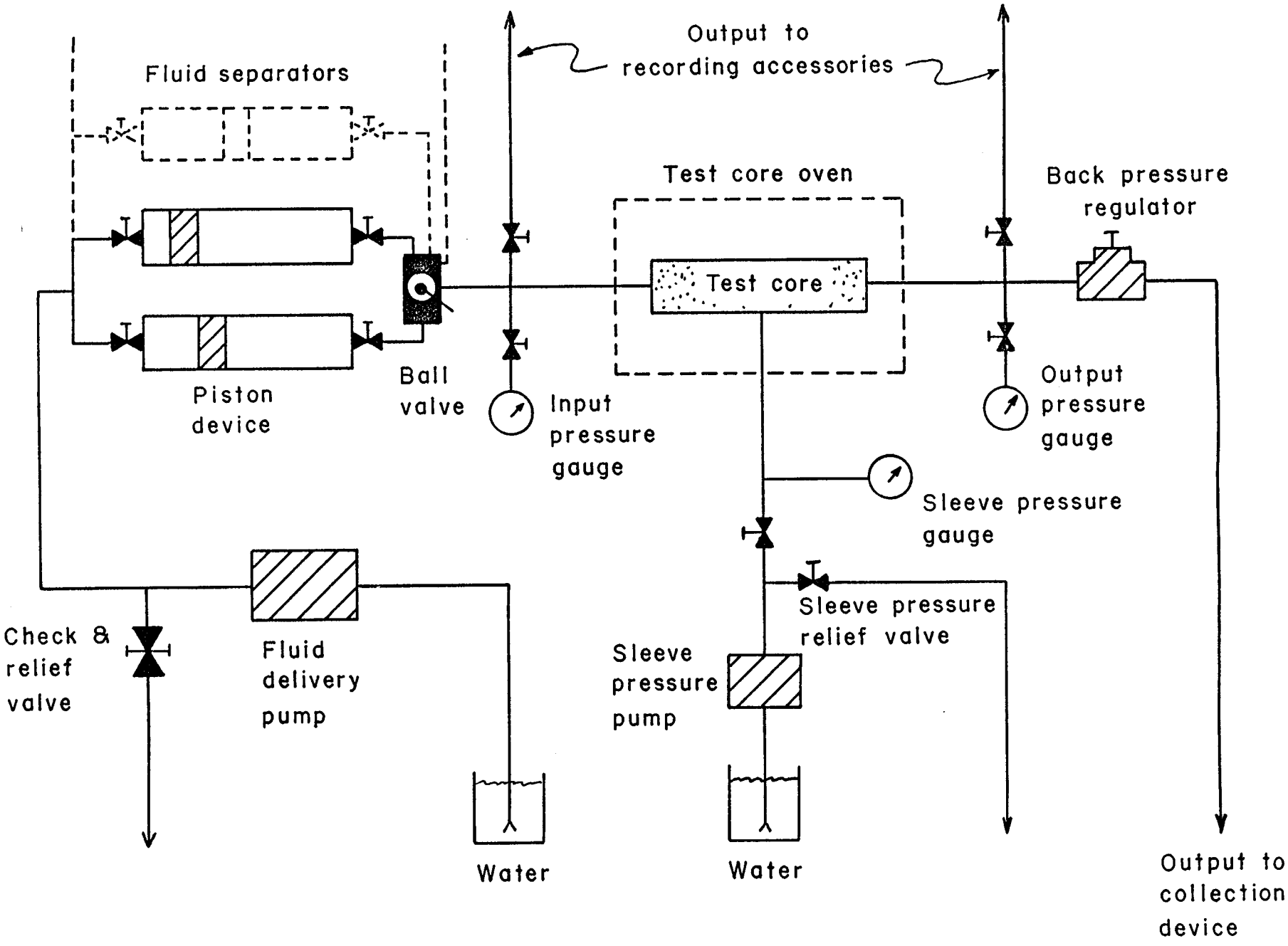

FIGURE 2. - Diagram of experimental core apparatus.

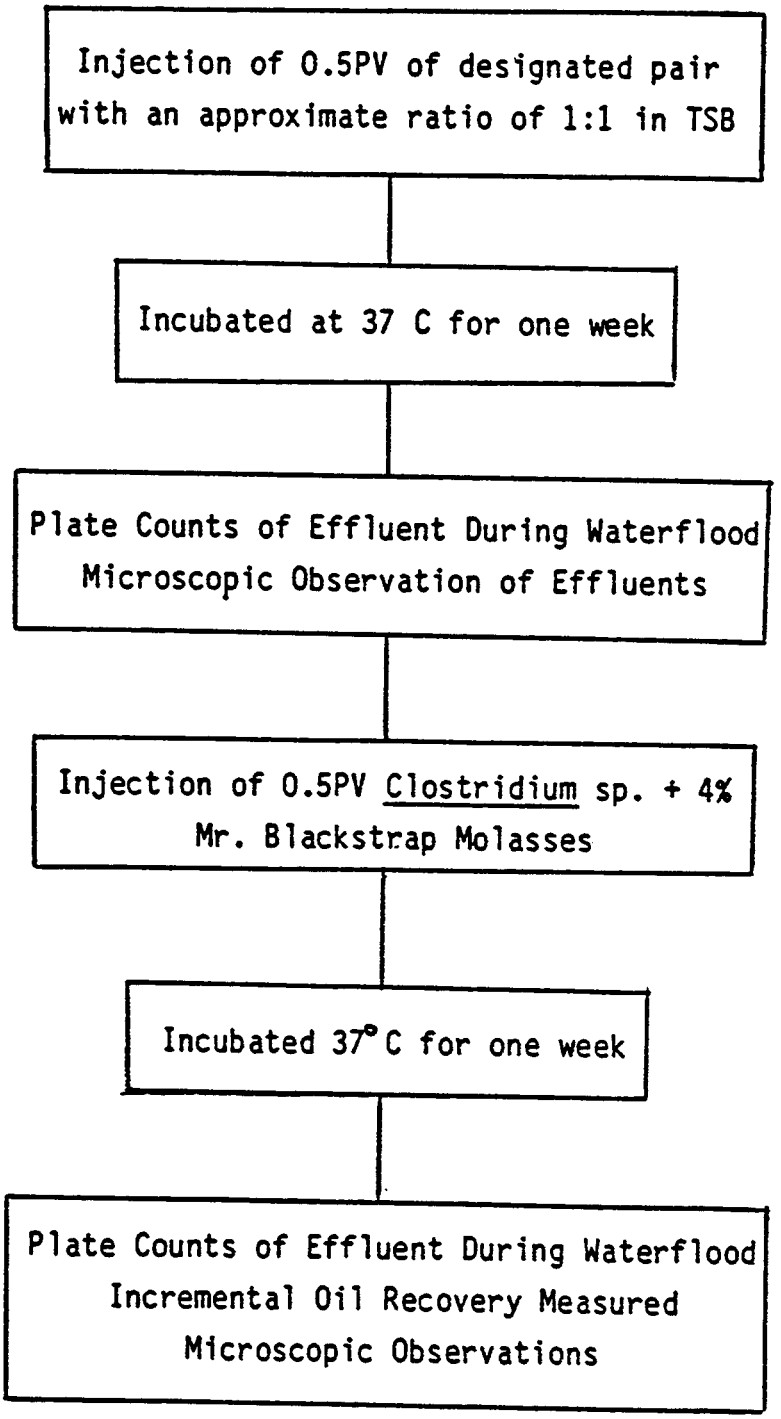

FIGURE 3. - Flowchart of experiments.

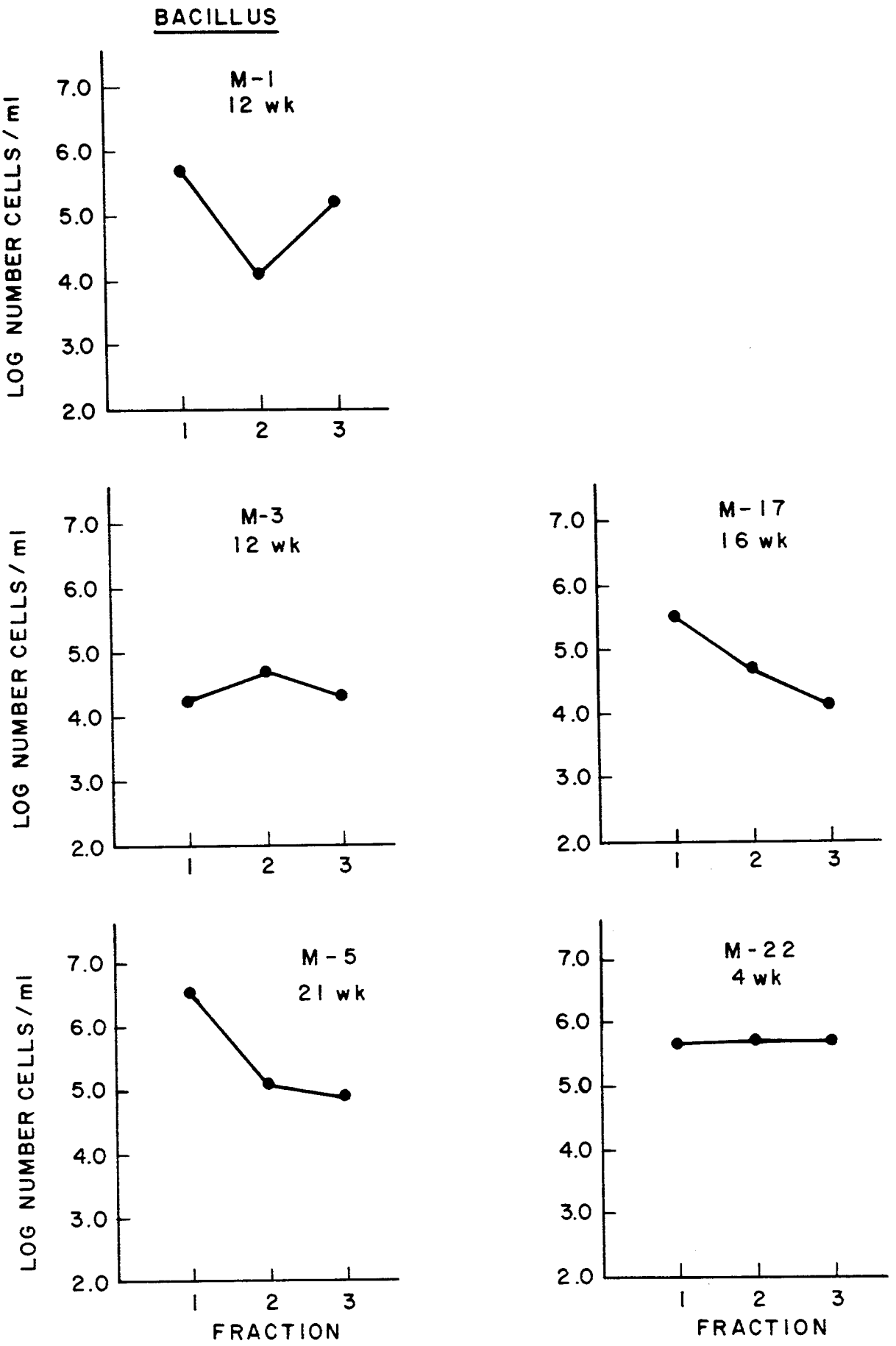

FIGURE 4. - Graphs of plate counts of effluents from Bacillus cores.

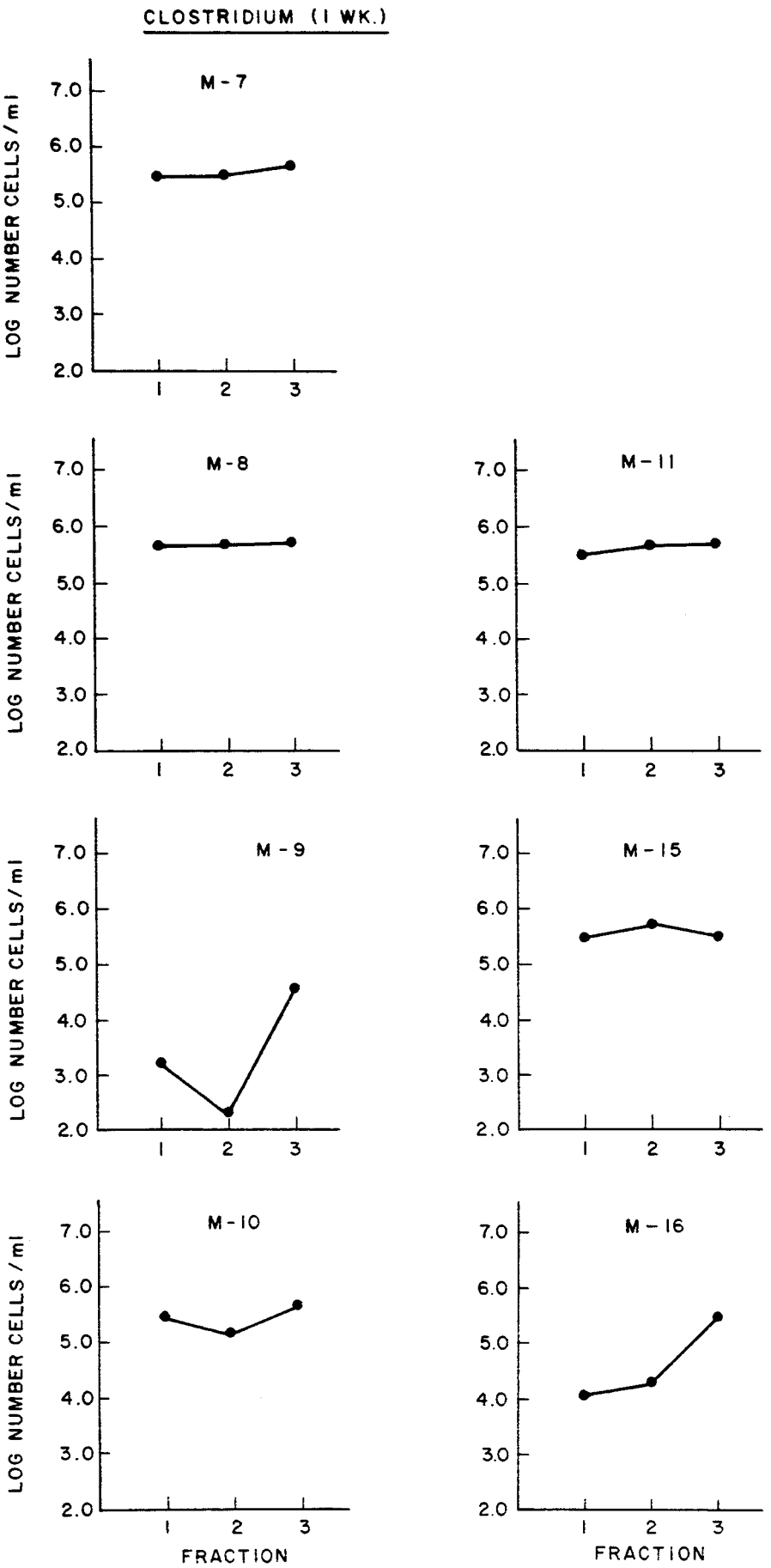

FIGURE 5. - Graphs of plate counts of effluents from Clostridium cores - one week incubations.

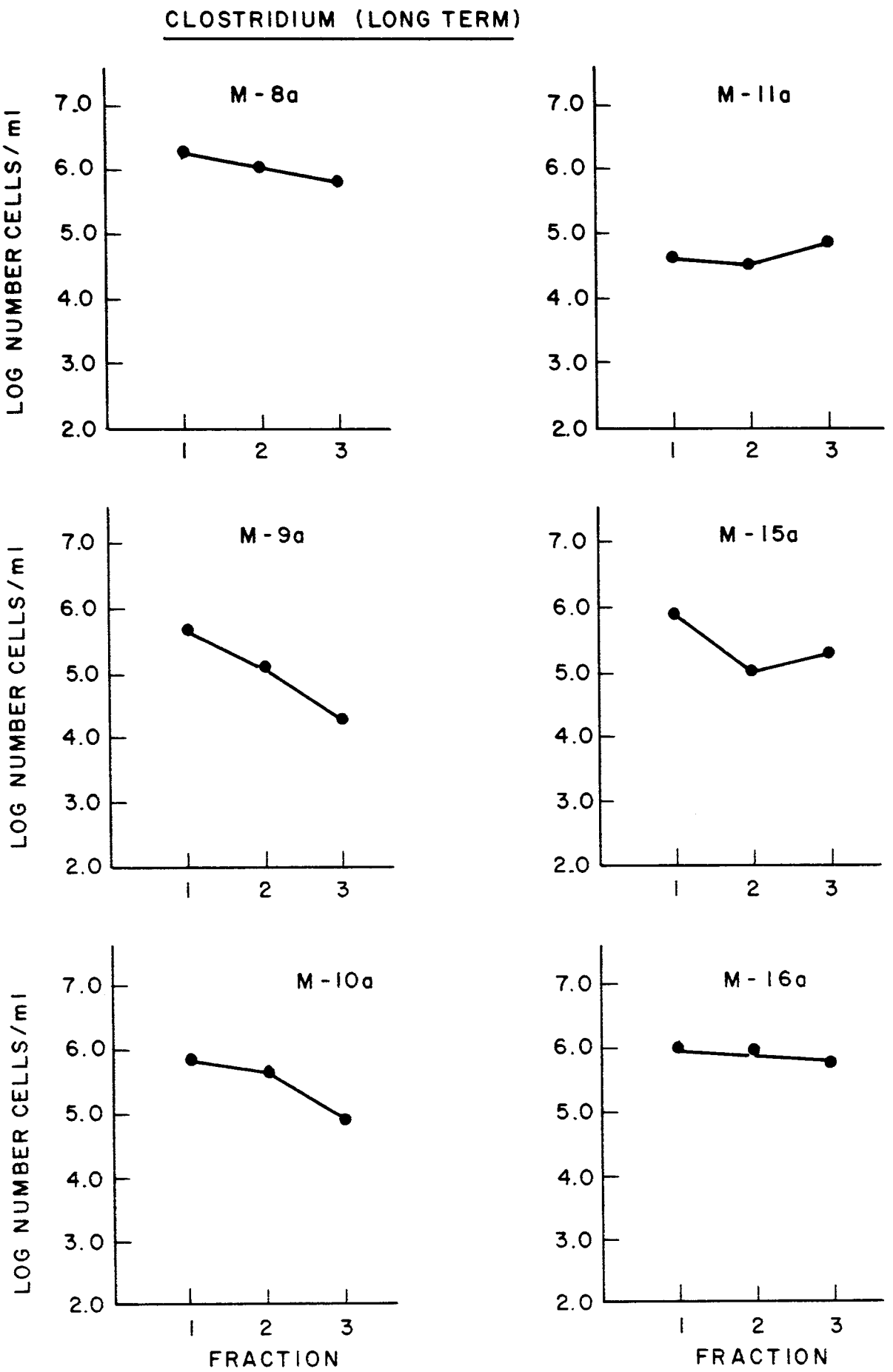

FIGURE 6. - Graphs of plate counts of effluents from Clostridium cores - long term incubations.

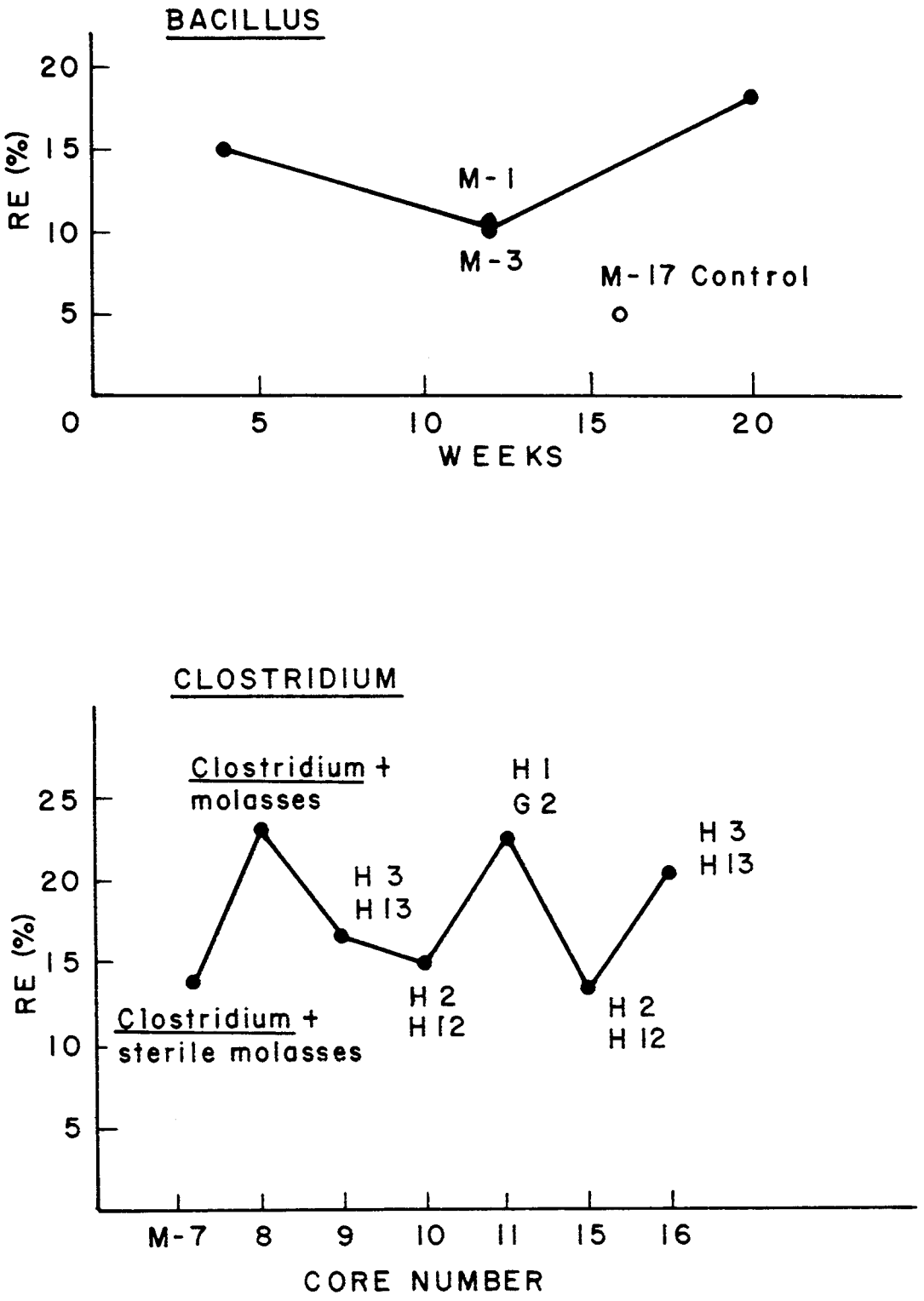

FIGURE 7. - Recovery efficiencies of Bacillus and Clostridium core experiments.

*U.S. GOVERNMENT PRINTING OFFICE: 1986-0-659-031/40,003